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(54) Title: FLOWERING TIME MODIFICATION

(57) Abstract: Recombinant polynucleotides and methods for modifying the flowering time of a plant are provided. Plants transformed with the recombinant polynucleotides may have flowering times that are accelerated, delayed or induced under specific conditions. Additionally, transformed plants may have altered vernalization requirements.

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FLOWERING TIME MODIFICATION

The present invention claims priority in part from US Provisional Application Serial Nos. 60/159,464 filed October 12, 1999; 60/164,132 filed November 8, 1999; 60/166,228 filed November 17, 1999; 60/197,899 filed April 17, 2000; and Plant Trait Modification III, filed August 22, 2000.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology and relates to compositions and methods for modifying a plant's flowering time or vernalization requirements.

BACKGROUND OF THE INVENTION

In order to maximize reproductive success, plants have evolved complex mechanisms to ensure that flowering occurs under favorable conditions. Analysis of late flowering mutants and ecotypes in *Arabidopsis* has revealed that such mechanisms are based upon several genetic pathways which may contain 80 or more genes (Martinez-Zapater and Somerville, (1990) *Plant Physiol.* 92:770-776; Koornneef et al. (1991) *Mol. Gen. Genet.* 229:57-66; EM Meyerwitz and CR Somerville Eds (1994) *Arabidopsis* pp 403-433 Cold Spring Harbor Laboratory Press, New York). Together these loci co-ordinate flowering time with environmental variables (e.g. day-length, temperature, light quality, and nutrient availability) and with the developmental stage of the plant.

Arabidopsis flowers rapidly when grown under long day conditions of 16 hours or continuous light, but flowers much later under short day conditions of 8 or 10 hours light. Genes regulating this response constitute the photoperiod pathway and were identified by mutations that cause late flowering under long day conditions but which do not alter flowering in short day conditions. Examples from this group, which promote flowering in response to long days, include CONSTANS (CO), GIGANTEA (GI), FT, FWA, FE, FD, and FHA. A second group of genes, which includes LUMINIDEPENDENS (LD), FCA, FVE, FY, and FPA, form an autonomous pathway that is active under all day-length conditions. Mutants for this second class of genes flower later than wild type controls irrespective of the day length conditions (Koornneef et al. (1991) Mol. Gen. Genet. 229:57-66; EM Meyerwitz and CR Somerville Eds (1994) Arabidopsis pp 403-433 Cold Spring Harbor Laboratory Press, New York).

In addition to differing in their response to day-length, mutants from the photoperiod and autonomous pathways show—a—differential response to prolonged cold (vernalization) treatments (Vince-Prue, (1975) *Vernalization*. In Photoperiodism in Plants pp 263-291, McGraw Hill, London) Through a vernalization response, *Arabidopsis* ecotypes from Northern

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latitudes, such as Stockholm, are adapted to flower in the spring following exposure to cold winter conditions. This avoids flowering in the late summer when seed maturation might be curtailed by the onset of winter conditions (Reeves and Coupland, (2000) *Curr. Opin. Plant Biol* 3:37-42). When these ecotypes are grown in the laboratory they flower late, but will flower much earlier if subjected to a cold period of 4-6 weeks during seed germination. In a comparable manner, mutants from the autonomous pathway exhibit a very marked reduction in flowering time when subjected to vernalization. In contrast, mutants from the photoperiod pathway only show a minor response to cold treatments (Chandler *et al.*, (1996) *Plant J.* 10:637-644; Koornneef *et al.*, (1998) *Genetics* 148:885-892). Thus, vernalization can overcome the requirement for the autonomous pathway conditions (Reeves and Coupland, (2000) *Curr. Opin. Plant Biol* 3:37-42).

Two Arabidopsis genes, FLOWERING LOCUS C, FLC (also known as FLOWERING LOCUS F, FLF) and FRIGIDA (FRI), act in conjunction to repress flowering in the absence of a vernalization treatment (Napp-Zinn, K. (1957) Indukt. Abstammungs. Verebungsl. 88:253-285; Napp-Zinn K. (1985) CRC Handbook of Flowering, Vol. 1, A. H. Halevy, pp 492-503; Clarke and Dean (1994) Mol. Gen. Genet. 248:81-89; Koornneef. et al., (1994) Plant Journal 6:911-919; Lee et al., (1994) Plant Journal 6:903-909.) Dominant functional alleles of FLC and FRI are found together in Northern European Arabidopsis ecotypes such as Pitztal and Stockholm. These ecotypes are extremely late flowering when non-vernalized. The widely used laboratory ecotype Columbia contains functional alleles at only one of these two loci and flower slightly later than strains such as Landsberg erecta which possess functional alleles of neither gene. The FRIGIDA protein sequence has not yet been published. However, the FLC gene has recently been cloned and shown to encode a MADS box protein (Sheldon C. et al., 1999, Plant Cell 11:445-458; Michaels S. and Amasino, R., 1999, Plant Cell 11:949-956). Dominant alleles and overexpression of FLC have been reported to delay flowering, while null flc mutants are early flowering (Lee et al., (1994) Plant J. 6:903-909; Michaels and Amasino, (1999) Plant Cell 11:949-956; Sheldon et al., (1999) Proc. Natl. Acad. Sci. 97:3753-3758). Thus, FLC acts to prevent premature flowering.

We have discovered transcription factors that regulate flowering time or vernalization requirements of plants. These transcription factors could therefore be useful to manipulate flowering characteristics of a plant.

SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a transgenic plant comprising a recombinant polynucleotide. The recombinant polynucleotide comprises a nucleotide sequence encoding a polypeptide comprising at least 6 consecutive amino acids of a

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sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28 but excluding SEQ ID No. 28, and the presence of the recombinant polynucleotide alters the flowering time or vernalization requirements of the transgenic plant when compared with the same trait of another plant lacking the recombinant polynucleotide.

In one embodiment, the nucleotide sequence encodes a polypeptide comprising a conserved domain such as 1) a localization domain, 2) an activation domain, 3) a repression domain, 4) an oligomerization domain or 5) a DNA binding domain of SEQ ID Nos. 2N, where N=1-28. In another embodiment, the recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28. In a further embodiment, the nucleotide sequence further comprises a promoter operably linked to the nucleotide sequence. The promoter may be a constitutive or inducible or tissue-active.

In a second aspect, the present invention relates to a method for altering a plant's flowering time or vernalization requirements. The method comprises (a) transforming a plant with a recombinant polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising at least 6 consecutive amino acids of a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28; (b) selecting transformed plants; and (c) identifying a transformed plant with the desired trait.

In one embodiment, the nucleotide sequence encodes a polypeptide comprising a conserved domain such as 1) a localization domain, 2) an activation domain, 3) a repression domain, 4) an oligomerization domain or 5) a DNA binding domain domain of SEQ ID Nos. 2N, where N=1-28 but excluding SEQ ID No. 28. In another embodiment, the recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28. In a further embodiment, the nucleotide sequence further comprises a promoter operably linked to the nucleotide sequence. The promoter may be a constitutive or inducible or tissue-active.

In a third aspect, the present invention relates to another method for altering a plant trait associated with flowering time or the plant's vernalization requirements. The method comprises (a) transforming the plant with a recombinant polynucleotide comprising a nucleotide sequence comprising at least 18 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID Nos. 2N-1, where N= 1-28 but excluding SEQ ID No. 27; and (b) selecting said transformed plant.

In yet another aspect, the present invention is yet another method for altering a plant's flowering time or vernalization requirements. The method comprises (a) providing a database sequence; (b) comparing the database sequence with a polypeptide selected from SEQ ID Nos. 2N, where N= 1-28; (c) selecting a database sequence that meets selected sequence

criteria; and (d) transforming said database sequence in the plant. Alternatively, the database sequence can be compared with a polynucleotide selected from SEQ ID Nos. 2N-1, where N= 1-28.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 2 provides a table of sequences that are homologous to the sequences provided in the Sequence Listing. The table includes from left to right: the SEQ ID No., the internal code reference number, the unique Genbank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

A "recombinant polynucleotide" is a nucleotide sequence comprising a gene coding sequence or a fragment thereof (comprising at least 18 consecutive nucleotides, preferably at least 30 consecutive nucleotides, and more preferably at least 50 consecutive nucleotides). Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker or the like. The polynucleotide may comprise single stranded or double stranded DNA or RNA. The polynucleotide may comprise modified bases or a modified backbone. The polynucleotide may be genomic, a transcript (such as an mRNA) or a processed nucleotide sequence (such as a cDNA). The polynucleotide may comprise a sequence in either sense or antisense orientations.

A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide is comprised of a nucleotide sequence not found in nature or the polynucleotide is separated from nucleotide sequences with which it typically is in proximity or is next to nucleotide sequences with which it typically is not in proximity.

A "recombinant polypeptide" is a polypeptide derived from the translation of a recombinant polynucleotide or is more enriched in a cell than the polypeptide in its natural

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state in a wild type cell, e.g. more than 5% enriched, more than 10% enriched or more than 20% enriched and is not the result of a natural response of a wild type plant or is separated from other components with which it is typically associated with in a cell.

A "transgenic plant" may refer to a plant that contains genetic material not normally found in a wild type plant of the same species, or in a naturally occurring variety or in a cultivar, and which has been introduced into the plant by human manipulation. A transgenic plant is a plant that may contain an expression vector or cassette. The expression cassette comprises a gene coding sequence and allows for the expression of the gene coding sequence. The expression cassette may be introduced into a plant by transformation or by breeding after transformation of a parent plant.

A transgenic plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells, protoplasts or any other plant material, and progeny thereof.

The phrase "altered expression" in reference to polynucleotide or polypeptide expression refers to an expression pattern in the transgenic plant that is different from the expression pattern in the wild type plant or a reference; for example, by expression in a cell type other than a cell type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern may be transient or stable, constitutive or inducible.

A "transcription factor" (TF) refers to a polynucleotide or polypeptide that controls the expression of a gene or genes either directly by binding to one or more nucleotide sequences associated with a gene coding sequence or indirectly by affecting the level or activity of other polypeptides that do bind directly or indirectly to one or more nucleotide sequences associated with a gene coding sequence. A TF, in this definition, includes any polypeptide that can activate or repress transcription of a single gene or a number of genes. This polypeptide group includes, but is not limited to, DNA binding proteins, protein kinases, protein phosphatases, GTP-binding proteins and receptors.

The transcription factor sequence may comprise a whole coding sequence or a fragment or domain of a coding sequence. A "fragment or domain", as referred to polypeptides, may be a portion of a polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner or to a similar extent as does the intact polypeptide. A fragment may comprise, for example, a DNA binding domain that binds to a specific DNA promoter region, an activation domain or a domain for protein-protein

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interactions. Fragments may vary in size from as few as 6 amino acids to the length of the intact polypeptide, but are preferably at least 30 amino acids in length and more preferably at least 60 amino acids in length. In reference to a nucleotide sequence "a fragment" refers to any sequence of at least consecutive 18 nucleotides, preferably at least 30 nucleotides, more preferably at least 50, of any of the sequences provided herein.

Exemplary polynucleotides and polypeptides comprise a sequence provided in the Sequence Listing as SEQ ID No. 1: G157 (cDNA); SEQ ID No. 2: G157 (protein); SEQ ID No. 3: G859 (cDNA); SEQ ID No. 4: G859 (protein); SEQ ID No. 5: G859.1 (cDNA); SEQ ID No. 6: G859.1 (protein); SEQ ID No. 7: G859.2 (cDNA); SEQ ID No. 8: G859.2 (protein); SEQ ID No. 9: G1842 (cDNA); SEQ ID No. 10: G1842 (protein); SEQ ID No. 11: G1842.2 (cDNA); SEQ ID No. 12: G1842.2 (protein); SEQ ID No. 13: G1842.6 (cDNA); SEQ ID No. 14: G1842.6 (protein); SEQ ID No. 15: G1842.7 (cDNA); SEQ ID No. 16: G1842.7 (protein); SEQ ID No. 17: G1843 (cDNA); SEQ ID No. 18: G1843 (protein); SEQ ID No. 19: G1844 (cDNA); SEQ ID No. 20: G1844 (protein); SEQ ID No. 21: G1844.2 (cDNA); SEQ ID No. 22: G1844.2 (protein); SEQ ID No. 23: G861 (cDNA); SEQ ID No. 24: G861 (protein); SEQ ID No. 25: G861.1 (cDNA); SEQ ID No. 26: G861.1 (protein); SEQ ID No. 27: G1759 (cDNA); SEQ ID No. 28: G1759 (protein); SEQ ID No. 29: G192 (cDNA); SEQ ID No. 30: G192 (protein); SEQ ID No. 31: G234 (cDNA); SEQ ID No. 32: G234 (protein); SEQ ID No. 33: G361 (cDNA); SEQ ID No. 34: G361 (protein); SEQ ID No. 35: G486 (cDNA); SEQ ID No. 36: G486 (protein); SEQ ID No. 37: G748 (cDNA); SEQ ID No. 38: G748 (protein); SEQ ID No. 39: G994 (cDNA); SEQ ID No. 40: G994 (protein); SEQ ID No. 41: G1335 (cDNA); SEQ ID No. 42: G1335 (protein); SEQ ID No. 43: G562 (cDNA); SEQ ID No. 44: G562 (protein); SEQ ID No. 45: G736 (cDNA); SEQ ID No. 46: G736 (protein); SEQ ID No. 47: G1073 (cDNA); SEQ ID No. 48: G1073 (protein); SEQ ID No. 49: G1435 (cDNA); SEQ ID No. 50: G1435 (protein); SEQ ID No. 51: G180 (cDNA); SEQ ID No. 52: G180 (protein); SEQ ID No. 53: G592 (cDNA); SEQ ID No. 54: G592 (protein); SEQ ID No. 55: G208 (cDNA); and SEQ ID No. 56: G208 (protein).

A "conserved domain" refers to a polynucleotide or polypeptide fragment that is more conserved at a sequence level than other fragments when the polynucleotide or polypeptide is compared with homologous genes or proteins from other plants. The conserved domain may be 1) a localization domain, 2) an activation domain, 3) a repression domain, 4) a dimerization or oligomerization domain, 5) a DNA binding domain or any combination thereof. For MADS proteins, the conserved domain is typically a DNA-binding domain.

A nucleotide sequence is "operably linked" when it is placed into a functional relationship with another nucleotide sequence. For example, a promoter or enhancer is operably linked to a gene coding sequence if the presence of the promoter or enhancer increases the level of expression of the gene coding sequence.

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"Trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. This characteristic may be visible to the human eye, such as seed or plant size, or be measured by biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes by employing Northerns, RT PCR, microarray gene expression assays or reporter gene expression systems or be measured by agricultural observations such as stress tolerance, yield or disease resistance.

"Trait modification" refers to a detectable difference in a characteristic in a transgenic plant with modified expression of a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. The trait modification may entail at least a 5% increase or decrease in an observed trait (difference), at least a 10% difference, at least a 20% difference, at least a 30%, at least a 50%, at least a 70%, at least a 100% or a greater difference. It is known that there may be a natural variation in the modified trait. Therefore, the trait modification observed entails a change in the normal distribution of the trait in transgenic plants compared with the distribution observed in wild type plant.

Trait modifications of particular interest include those to seed (embryo), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; enhanced resistance to microbial, fungal or viral diseases; resistance to nematodes, decreased herbicide sensitivity, enhanced tolerance of heavy metals (or enhanced ability to take up heavy metals), enhanced growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotypes that may be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenyllipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that may be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that may be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time, flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

Of particular interest are traits relating to modified vernalization requirements or flowering time characteristics, such as changes in flowering time in response to day-length, in response to temperature, in response to light quality, nutrient availability, and development stage of the plant, and the like.

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1. The Sequences

We have discovered particular plant transcription factors (TFs) that can be employed to modify the flowering time of a plant. Therefore, the flowering time of plants can be either decreased, increased, or made inducible under specific conditions using the TFs of this invention. Additionally, the transcription factors can be used to modify the vernalization requirements of the plant.

The plant transcription factors may belong to one of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) Biol. Chem. 379:633-646); the MYB transcription factor family (Martin and Paz-Ares, (1997) Trends Genet. 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) Biol. Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4:1575-1588); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes. Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 1996 250:7-16); the NAM protein family (Souer et al. (1996) Cell 85:159-170); the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNAbinding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the Box P-binding protein (the BPF-1) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); the high mobility group (HMG) family (Bustin and Reeves (1996) Prog. Nucl. Acids Res. Mol. Biol. 54:35-100); the scarecrow (SCR) family (Di Laurenzio et al. (1996) Cell 86:423-433); the GF14 family (Wu et al. (1997) Plant Physiol. 114:1421-1431); the polycomb (PCOMB) family (Kennison (1995) Annu. Rev. Genet. 29:289-303); the teosinte branched (TEO) family (Luo et al. (1996) Nature 383:794-799; the ABI3 family (Giraudat et al. (1992) Plant Cell 4:1251-1261); the triple helix (TH) family (Dehesh et al. (1990) Science 250:1397-1399); the EIL family (Chao et al. (1997) Cell 89:1133-44); the AT-HOOK family (Reeves and Nissen (1990) Journal of Biological Chemistry 265:8573-8582); the S1FA family (Zhou et al. (1995) Nucleic Acids Res. 23:1165-1169); the bZIPT2 family (Lu and Ferl (1995) Plant Physiol. 109:723); the YABBY family (Bowman et al. (1999) Development 126:2387-96); the PAZ family (Bohmert et al. (1998)

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EMBO J. 17:170-80); a family of miscellaneous (MISC) transcription factors including the DPBF family (Kim et al. (1997) *Plant J.* 11:1237-1251) and the SPF1 family (Ishiguro and Nakamura (1994) *Mol. Gen. Genet.* 244:563-571); the golden (GLD) family (Hall et al. (1998) *Plant Cell* 10:925-936), and the TUBBY family (Boggin et al, (1999) Science 286:2119-2125)

In particular, the TFs that we have discovered that are implicated in flowering time or vernalization include members of the MADS transcription factor family, the MYB family, the WRKY family, the HLH/MYC family, GLD family, AT-HOOK family, the CAAT family, the bZIP family, and members of zinc coordinating protein families (Z-Dof, Z-CLDSH and Z-CH2H2). In fact we have identified the first members of the WRKY, CAAT, bZIP, AT-HOOK and HLH/MYC families that are associated with flowering time modification in plants: G192 and G190 (WRKY), G486 (CAAT), G562 (bZIP), G1073 (AT-HOOK) and G592 (HLH/MYC).

The polynucleotides and polypeptides are provided in the Sequence Listing and are tabulated in Figure 1. Figure 1 identifies a SEQ ID No., its corresponding GID number, whether the sequence is a polynucleotide or a polypeptide sequence, and indicates the conserved domains. We have also identified domains or fragments derived from each of the sequences in the Sequence Listing. The fragments can be from any region of the sequence, can be of any length up to the length of the sequence, and can be as short as six residues for protein and 18 nucleotides for DNA. Exemplary fragments of the DNA sequences are as follows: 1-50, 51-100, 101-200, 201-218, 218-300, 301-450 and 450-600; and exemplary fragments of proteins are as follows 1-50, 51-100, 101-200, 201-206, 206-250, 251-300. For DNA sequences, the numbers may be measured from either 5' or 3' end of the DNA. For the protein sequences the fragment location is determined from the N-terminus or C-terminus of the protein and may include adjacent amino acid sequences, such as for example for SEQ ID No. 2 an additional 10, 20, 40, 60 or 100 amino acids in either N-terminal or C-terminal direction of the described fragments.

The identified polypeptide fragments may be linked to fragments or sequences derived from other transcription factors so as to generate additional novel sequences, such as by employing the methods described in Short, PCT publication WO9827230, entitled "Methods and Compositions for Polypeptide Engineering" or in Patten et al., PCT publication WO9923236, entitled "Method of DNA Shuffling" or in Minshull and Stemmer, US Patent No. 5,837,458. Alternatively, the identified fragment may be linked to a transcription activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. A common feature of some activation domains is that they are designed to form amphiphilic alpha helices with excess positive or negative charge (Giniger and Ptashne (1987) Nature 330:670-672, Gill and Ptashne (1987) Cell 51:121-126, Estruch et al (1994) Nucl. Acids Res. 22:3983-3989). Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al.

(1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51; 113-119) and synthetic peptides (Giniger and Ptashne, supra).

The isolated polynucleotides and polypeptides may be used to modify plant development, physiology or biochemistry such that the modified plants have a trait advantage over wild type plants. The identified polynucleotide fragments are also useful as nucleic acid probes and primers. A nucleic acid probe is useful in hybridization protocols, including protocols for microarray experiments. Primers may be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook et al., *Molecular Cloning. A Laboratory Manual*, Ed. 2, Cold Spring Harbor Laboratory Press, New York (1989) and Ausubel et al. (eds) *Current Protocols in Molecular Biology*, John Wiley & Sons (1998).

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2. Identification of Homologous Sequences (Homologs)

Homologous sequences to those provided in the Sequence Listing derived from *Arabidopsis thaliana* or from other plants may be used to modify a plant trait. Homologous sequences may be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype may be changed include barley, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, sweet potato and beans. The homologs may also be derived from woody species, such pine, poplar and eucalyptus.

Substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) *Meth. Enzymol.* (1993) vol. 217, Academic Press). Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are

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made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure.

Substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions may be conservative with little effect on the function of the gene, for example by substituting alanines for serines, arginines for lysines, glutamate for aspartate and the like. The substitutions which are not conservative are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

Additionally, the term "homologous sequence" may encompass a polypeptide sequence that is modified by chemical or enzymatic means. The homologous sequence may be a sequence modified by lipids, sugars, peptides, organic or inorganic compounds, by the use of modified amino acids or the like. Protein modification techniques are illustrated in Ausubel et al. (eds) *Current Protocols in Molecular Biology*, John Wiley & Sons (1998).

Homologous sequences also may mean two sequences having a substantial percentage of sequence identity after alignment as determined by using sequence analysis programs for database searching and sequence alignment and comparison available, for example, from the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madision, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) may be searched. Alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window may be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous

positions. A description of the method is provided in Ausubel et al. (eds) (1999) *Current Protocols in Molecular Biology*, John Wiley & Sons.

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Transcription factors that are homologs of the disclosed sequences will typically share at least 40% amino acid sequence identity. More closely related TFs may share at least 50%, 60%, 65%, 70%, 75% or 80% sequence identity with the disclosed sequences. Factors that are most closely related to the disclosed sequences share at least 85%, 90% or 95% sequence identity. At the nucleotide level, the sequences will typically share at least 40% nucleotide sequence identity, preferably at least 50%, 60%, 70% or 80% sequence identity, and more preferably 85%, 90%, 95% or 97% sequence identity. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein.

One way to identify whether two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions. Generally, stringent conditions are selected to be about 5°C to 20°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook et al. (1989) *Molecular Cloning. A Laboratory Manual*, Ed. 2, Cold Spring Harbor Laboratory Press, New York and Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* Part I, Elsevier, New York. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For detecting less closely related homologs washes may be performed at 50° C.

For conventional hybridization the hybridization probe is conjugated with a detectable label such as a radioactive label, and the probe is preferably of at least 20 nucleotides in length. As is well known in the art, increasing the length of hybridization probes tends to give enhanced specificity. The labeled probe derived from the *Arabidopsis* nucleotide sequence may be hybridized to a plant cDNA or genomic library and the hybridization signal detected using means known in the art. The hybridizing colony or plaque (depending on the type of library used) is then purified and the cloned sequence contained in that colony or plaque isolated and characterized. Homologs may also be identified by PCR-based techniques, such as inverse PCR or RACE, using degenerate primers. See Ausubel et al. (eds) (1998) *Current Protocols in Molecular Biology*, John Wiley & Sons.

TF homologs may alternatively be obtained by immunoscreening an expression library. With the provision herein of the disclosed TF nucleic acid sequences, the polypeptide may be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise

antibodies (monoclonal or polyclonal) specific for the TF. Antibodies may also be raised against synthetic peptides derived from TF amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone the TF homolog, using the methods described above. The selected cDNAs may be confirmed by sequencing and biological activity.

3. Altered Expression of Transcription Factors

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Any of the identified sequences may be incorporated into a cassette or vector for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described including those described in Weissbach and Weissbach, (1989) *Methods for Plant Molecular Biology*, Academic Press, and Gelvin et al., (1990) *Plant Molecular Biology Manual*, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella, L., et al., (1983) *Nature* 303: 209, Bevan, M., *Nucl. Acids Res.* (1984) 12: 8711-8721, Klee, H. J., (1985) *Bio/Technology* 3: 637-642, for dicotyledonous plants. Ti-derived plasmids can be transferred into both monocotonous and docotyledonous species using Agrobacterium-mediated transformation (Ishida et al (1996) *Nat. Biotechnol.* 14:745-50; Barton et al. (1983) *Cell* 32:1033-1043).

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Alternatively, non-Ti vectors can be used to transfer the DNA into plants and cells by using free DNA delivery techniques. Such methods may involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide wiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou, P., (1991) *Bio/Technology* 9: 957-962) and corn (Gordon-Kamm, W., (1990) *Plant Cell* 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks, T. et al., (1993) *Plant Physiol.* 102: 1077-1084; Vasil, V., (1993) *Bio/Technology* 10: 667-674; Wan, Y. and Lemeaux, P., (1994) *Plant Physiol.* 104: 37-48, and for *Agrobacterium*-mediated DNA transfer (Ishida et al., (1996) *Nature Biotech.* 14: 745-750).

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Typically, plant transformation vectors include one or more cloned plant coding sequences (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

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Examples of constitutive plant promoters which may be useful for expressing the TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (see, e.g., Odel et al., (1985) Nature 313:810); the nopaline synthase promoter (An et al., (1988) Plant Physiol. 88:547); and the octopine synthase promoter (Fromm et al., (1989) Plant Cell 1: 977).

A variety of plant gene promoters that regulate gene expression in response to environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of the TFs in plants, as illustrated by seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186; fruit-specific promoters that are active during fruit ripening (such as the dru 1 promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No. 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol. Biol. 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929). promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol. Biol. 37:977-988), flower-specific (Kaiser et al, (1995) Plant Mol. Biol. 28:231-243), pollen (Baerson et al. (1994) Plant Mol. Biol. 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson et al. (1993) Plant Mol. Biol. 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al (1999) Plant Mol. Biol. 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) Plant Mol. Biol. 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol. Biol. 38:1053-1060, Willmott et al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley, et al. (1993) Plant Mol. Biol. 22: 13-23), light (e.g., the pea rbcS-3A promoter, Kuhlemeier et al., (1989) Plant Cell 1:471, and the maize rbcS promoter, Schaffner and Sheen, (1991) Plant Cell 3: 997); wounding (e.g., wunl, Siebertz et al., (1989) Plant Cell 1: 961); pathogen resistance, and chemicals such as methyl jasmonate or salicylic acid.(Gatz et al., (1997) Plant Mol. Biol. 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at late seed development (Odell et al. (1994) Plant Physiol. 106:447-458).

Plant expression vectors may also include RNA processing signals that may be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors may include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

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Finally, as noted above, plant expression vectors may also include dominant selectable marker genes to allow for the ready selection of transformants. Such genes include those encoding antibiotic resistance genes (e.g., resistance to hygromycin, kanamycin, bleomycin, G418, streptomycin or spectinomycin) and herbicide resistance genes (e.g., phosphinothricin acetyltransferase).

A reduction of TF expression in a transgenic plant to modify a plant trait may be obtained by introducing into plants antisense constructs based on the TF cDNA. For antisense suppression, the TF cDNA is arranged in reverse orientation relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length TF cDNA or gene, and need not be identical to the TF cDNA or a gene found in the plant type to be transformed. Generally, however, where the introduced sequence is of shorter length, a higher degree of homology to the native TF sequence will be needed for effective antisense suppression. Preferably, the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous TF gene in the plant cell. Suppression of endogenous TF gene expression can also be achieved using a ribozyme. Ribozymes are synthetic RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 to Cech and U.S. Patent No. 5.543.508 to Haselhoff. The inclusion of ribozyme sequences within antisense RNAs may be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that bind to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Vectors in which RNA encoded by the TF cDNA (or variants thereof) is over-expressed may also be used to obtain co-suppression of the endogenous TF gene in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire TF cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous TF gene. However, as with antisense suppression, the suppressive efficiency will be enhanced as (1) the introduced sequence is lengthened and (2) the sequence similarity between the introduced sequence and the endogenous TF gene is increased.

Vectors expressing an untranslatable form of the TF mRNA may also be used to suppress the expression of endogenous TF activity to modify a trait. Methods for producing such constructs are described in U.S. Patent No. 5,583,021 to Dougherty et al. Preferably, such constructs are made by introducing a premature stop codon into the TF gene. Alternatively, a

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plant trait may be modified by gene silencing using double-strand RNA (Sharp (1999) *Genes and Development* 13: 139-141). This approach, whereby a vector is prepared in which a cDNA or gene is arranged in duplicated fashion and is capable of generating upon expression a double stranded RNA molecule with a hairpin structure. This procedure has been used to modify gene activity in plants (Chuang and Meyerowitz (1999) *Proc. Natl. Acad. Sci.* 97:4985-9490).

Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a TF gene. Mutants containing a single mutation event at the desired gene may be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) *Methods in Arabidopsis Research*. World Scientific).

A plant trait may also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome may be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention may also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al., (1997) *Nature* 390 698-701, Kakimoto et al., (1996) *Science* 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant may be modified so as to increase transcription levels of a polynucleotide of the invention (See PCT Publications WO9606166 and WO 9853057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant may also comprise the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

4. Transgenic Plants with Modified TF Expression

Once an expression cassette comprising a polynucleotide encoding a TF gene of this invention has been constructed, standard techniques may be used to introduce the polynucleotide into a plant in order to modify a trait of the plant. The plant may be any higher plant, including gymnosperms, monocotyledonous and dicotyledenous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean, clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcurbitaceae*

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(melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) *Handbook of Plant Cell Culture –Crop Species.* Macmillan Publ. Co. Shimamoto et al. (1989) *Nature* 338:274-276; Fromm et al. (1990) *Bio/Technology* 8:833-839; and Vasil et al. (1990) *Bio/Technology* 8:429-434.

Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods may include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumeficiens* mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait may be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention may be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

5. Commercial Applications of the Polynucleotides and Polypeptides

Specific applications for the genes of the present invention relate to their potential roles in plant flowering time or the vernalization response. Most modern crop varieties are the result of extensive breeding programs and many generations of backcrossing may be required

to introduce desired traits. Systems that accelerate flowering could have valuable applications in such programs since they allow much faster generation times. Additionally, in some instances, a faster generation time might allow additional harvests of a crop to be made within a given growing season. With the advent of transformation systems for tree species such as oil palm, aspen, pine and eucalyptus, forest biotechnology is a growing area of interest.

Also, in species such as sugarbeet where the vegetative parts of the plants constitute the crop and the reproductive tissues are discarded, it would be advantageous to delay or prevent flowering. Extending vegetative development could bring about large increases in yields.

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Furthermore, by regulating the expression of flowering-time controlling genes, using inducible promoters, flowering could potentially be triggered as desired (for example, by application of a chemical inducer). This would allow, for example, flowering to be synchronized across a crop and facilitate more efficient harvesting. Such inducible systems could be used to tune the flowering of crop varieties to different latitudes. At present, species such as soybean and cotton are available as a series of maturity groups that are suitable for different latitudes on the basis of their flowering time (which is governed by day-length). A system in which flowering could be chemically controlled would allow a single high-yielding northern maturity group to be grown at any latitude. In southern regions such plants could be grown for longer, thereby increasing yields, before flowering was induced. In more northern areas, the induction would be used to ensure that the crop flowers prior to the first winter frosts. Currently, the existence of a series of maturity groups for different latitudes represents a major barrier to the introduction of new valuable traits.

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For many crop species, high yielding winter-varieties can only be grown in temperate regions where the winter season is prolonged and cold enough to elicit a vernalization response. Altered expression of the genes of the invention could compensate for a vernalization treatment in late-flowering *Arabidopsis* ecotypes. Similar effects might be achieved in crop plants. Winter varieties of wheat, for instance, which over-express G157 (or the wheat ortholog) might then be grown in areas like Southern California which would otherwise be too warm to allow effective vernalization. A second application for this system is in cherry (*Prunus*). Locally grown cherries are unavailable in the early Californian spring since the winters are too warm for vernalization to occur.

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A further application exists in strawberry (*Fragaria*). Strawberry has a well-defined perennial cycle of flower initiation, dormancy, chilling, crop growth and runner production. In temperate European countries, the plants flower in early spring, and fruit is produced in May or June. Following fruiting, runners are generated that carry plantlets which take root. The plants then remain dormant all through the late summer and autumn. Flowering cannot be repeated until the following spring after the plants have received a winter cold treatment. A

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system, which bypasses this vernalization requirement, might permit a second autumn crop of strawberries to be harvested in addition to the spring crop.

Finally, in addition to the direct applications of the genes themselves, their regulatory regions could also be of value. If the promoters of these genes are responsive to low temperatures they could be incorporated into expression systems for regulation of genes that confer tolerance to freezing. Such genes would then be up regulated specifically at the time required, thereby minimizing any toxic effects that result from their constitutive expression.

6. Other Utility of the Polypeptide and Polynucleotides

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A transcription factor coding provided by the present invention may also be used to identify exogenous or endogenous molecules that may affect expression of the transcription factors and may affect flowering time. These molecules may include organic or inorganic compounds.

For example, the method may entail first placing the molecule in contact with a plant or plant cell. The molecule may be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide may be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence may be detected by use of microarrays, Northerns or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) *Current Protocols in Molecular Biology*, John Wiley & Sons (1998). Such changes in the expression levels may be correlated with modified plant traits and thus identified molecules may be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

The transcription factors may also be employed to identify promoter sequences with which they may interact. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence may be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences may be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the TFs with their promoters (Bulyk et al. (1999) *Nature Biotechnology* 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification may occur by covalent modification, such

as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any method suitable for detecting protein-protein interactions may be employed. Among the methods that may be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

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The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA, 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast Saccharomyces cerevisiae that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions may be preformed.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLES

Methods

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All experiments were performed using *Arabidopsis* of ecotype Columbia except where otherwise indicated. The Stockholm (CS6863) and Pitztal (CS6832) lines were supplied by the ABRC at Ohio State University. In all experiments, seeds were sterilized by a 2 minute ethanol treatment followed by 30 minutes in 30% bleach / 0.01% Tween and five washes in distilled water. Seeds were sown to MS agar in 0.1% agarose and stratified for 3-5 days at 4 °C, before transfer to growth rooms with a temperature of 20-25 °C. MS media was supplemented with 50mg/l kanamycin for selection of transformed plants. Plants were transplanted to soil after 7 days of growth on plates. For vernalization treatments, seeds were sown to MS agar plates, sealed with micropore tape, and placed in a 4°C cold room with low light levels for 6-8 weeks. The plates were then transferred to the growth rooms alongside plates containing freshly sown non-vernalized controls. Whole vegetative seedlings were harvested for gene expression analysis at 6 to 9 days after transfer. Rosette leaves were counted when a visible inflorescence of approximately 3 cm was apparent. Rosette and total

leaf number on the progeny stem are tightly correlated with the timing of flowering (Koornneef et al (1991) *Mol. Gen. Genet* 229:57-66.

Example I. Full Length Gene Identification and Cloning

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For the following examples, G157 refers to SEQ ID Nos 1 and 2, G859 refers to SEQ ID Nos. 3-8, G1842 refers to SEQ ID Nos. 9-16, G1843 refers to SEQ ID Nos. 17 and 18, G1844 refers to SEQ ID Nos. 19-22, G861 refers to SEQ ID Nos. 23-26 and FLC or G1759 refers to SEQ ID Nos. 27, 28.

Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of –4 or –5 or lower, depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

For example, we identified a MADS box gene G157 within BAC F22K20 (GenBank accession AC002291) from Chromosome 1 that was predicted to encode a protein related to FLC. An 872bp cDNA clone for G157 was identified among clones isolated from a library derived from leaf mRNA. The encoded protein was 196 amino acids in length, and shared 62% overall amino acid sequence identity with FLC, and 82% identity within the MADS DNA binding domain.

G157 is also related to G859, G1842, G1843, and G1844 that map together as a tightly linked cluster, at the bottom of chromosome V, that occupies approximately 22 kb and spans three adjacent clones, MXK3, F15O5, and MQN23 (GenBank accession numbers AB019236, AB026633, and AB013395, respectively). G859, G1842, G1843, and G1844 are all arranged in the same orientation. G859, G1842, G1843, and G1844 were likely created by a duplication event; this could have allowed their divergence into different aspects of gene regulation. Their physical proximity suggests that they may act as a unit controlled via common regulatory elements.

The pair-wise comparisons of the 57 amino acid MADS domains of FLC, G157, G859, G1842, G1843, and G1844 are displayed in Table 1. The table shows percent amino acid sequence identity and, in parentheses, the sequence identity percentages when conservative amino acid substitutions are considered. The MADS domains of the proteins encoded by G859, G1842, G1843, and G1844 are highly conserved with those of FLC and G157: these proteins share from 75% to 91% of amino acid sequence identity, depending on the pair-wise comparison as shown below. When conservative amino substitutions are made, the MADS domains of these proteins are 88%-99% identical to each other (shown in parentheses).

Table 1 Percentage of amino acid identity in the MADS domain

	FLC (G1759)	G157	G859	G1842	G1843	G1844
FLC (G1759)	100%	82%(96%)	84%(94%)	77%(91%)	78%(99%)	75%(92%)
G157	-	100%	87%(95%)	89%(94%)	78%(95%)	78%(93%)
G859	-	-	100%	91%(94%)	77%(94%)	78%(92%)
G1842	-	-	-	100%	77%(91%	78%(88%)
G1843	-	-	-	-	100%	85%(92%)
G1844	-	-	-	-	-	100%

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Amino acid residue 30 of FLC and by G157, G859, G1842, G1843, and G1844 is an acidic residue (E or D) whereas, in all other *Arabidopsis* MADS domain proteins so far identified, that position is occupied by a positively charged lysine residue. The crystal structure of the human SRF MADS domain bound to DNA has shown that lysine residue (which is also conserved in yeast MCM1 and human MEF2A proteins) to contact the phosphate backbone of the DNA target site (Pellegrini *et al.*, (1995) Nature 376:490-498). That amino acid difference could therefore confer DNA binding properties to FLC and by G157, G859, G1842, G1843, and G1844 distinct from other *Arabidopsis* MADS domain proteins. Therefore, MADS domain proteins with an acidic residue at position 30 may be particularly useful in modifying plant flowering time and vernalization response.

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The transcripts from these genes were analyzed by 3' RACE (Rapid Amplification cDNA Ends) and corresponding cDNAs were isolated by RT-PCR from mixed samples of *Arabidopsis* tissue (Columbia ecotype). During this analysis, it was found that G859, G1842 and G1844 transcripts exist in multiple alternatively spliced forms.

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Example II. Flowering Time Associated Genes

Reverse transcriptase PCR was done using gene specific primers within the coding region for each sequence identified. Where possible, the primers were designed near the 3' region of each coding sequence initially identified.

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Total RNA was isolated from plant tissue tissue and extracted using CTAB. Once extracted total RNA was normalized in concentration across all the tissue types to ensure that the PCR reaction for each tissue received the same amount of cDNA template using the 28S band as reference. Poly A+ was purified using a modified protocol from the Qiagen Oligotex kit batch protocol. cDNA was synthesized using standard protocols. After the first strand cDNA synthesis, primers for Actin 2 were used to normalize the concentration of cDNA across the tissue types. Actin 2 is found to be constitutively expressed in fairly equal levels across the Arabidopsis tissue types.

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For RT PCR, cDNA template was mixed with corresponding primers and Taq polymerase. Each reaction consisted of 0.2 ul cDNA template, 2ul 10X Tricine buffer, and

16.8 ul water, 5pmol Primer 1, 5pmol Primer 2, 0.3 ul Taq polymerase, 200uM dNTPs and 8.6 ul water.

The 96 well plate was covered with microfilm and set in the Thermocycler to start the following reaction cycle. Step1 93° C for 3 mins, Step 2 93° C for 30 sec, Step 3 60-65° C for 1 min, Step 4 72° C for 2 mins,. Steps 2, 3 and 4 were repeated for 20-35 cycles, Step 5 72° C for 5 mins and Step 6 4° C. The PCR plate was sometimes placed back in the thermocycler to amplify more products for 5-15 more cycles to identify genes that have very low expression. The reaction cycle was as follows: Step 2 93° C for 30 sec, Step 3 65° C for 1 min, and Step 4 72° C for 2 ins, repeated for 8 cycles, and Step 4 4° C.

Eight microliters of PCR product and 1.5 ul of loading dye were loaded on a 1.2% agarose gel for analysis between 21 and 36 cycles. Expression levels of specific transcripts were considered low if they were only detectable after 35 cycles of PCR. Expression levels were considered medium or high depending on the levels of transcript compared with observed transcript levels for actin2.

As an example, to assess G157 mRNA levels in G157 plants, PCR was carried out over 25 cycles using primers 5'-GGCATAACCCTTATCGGAGATTTGAAGC-3' (SEQ ID No. 57) and 5'-ACACAAACTCTGATCTTGTCTCCGAAGG-3' (SEQ ID No. 58). To assess mRNA levels in different tissues extracted from wild type plants, 25 or 30 cycles of PCR were performed using primers 5'-GCATAACCCTTATCGGAGATTTGAAGCCAT-3' (SEQ ID No. 59) and 5'-AACATTCCTCTCTCATCATCTGTTGCCAGC-3' (SEQ ID No. 60). PCR for FLC was performed either with primers 5'-AACGCTTAGTATCTCCGGCGACTTGAAC-3' (SEQ ID No. 51) and 5'-CTCACACGAATAAGGTACAAAGTTCATC-3' (SEQ ID No. 62) over 35 cycles, or 5'-TTAGTATCTCCGGCGACTTGAACCCAAACC-3' (SEQ ID No. 63) 5'-AGATTCTCAACAAGCTTCAACATGAGTTCG-3' (SEQ ID No. 64) over 30 cycles. Primer specificity was verified by sequencing RT-PCR products. Samples were standardized via 20-25 cycles of PCR with actin primers.

Example III. Construction of Expression Vectors

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The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) *Nucleic Acids Research* 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with Sall and Notl restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid

were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l spectinomycin (Sigma).

Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l spectinomycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini Prep kits (Qiagen, CA).

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Example IV. Transformation of Agrobacterium with the Expression Vector

After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of *Agrobacterium tumefaciens* cells for transformation were made as described by Nagel et al. *FEMS Microbiol Letts* 67: 325-328 (1990). *Agrobacterium* strain GV3101 was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance (A_{600}) of 0.5-1.0 was reached. Cells were harvested by centrifugation at $4,000 \times g$ for 15 min at 4° C. Cells were then resuspended in 250 µl chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125 µl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 µl and 750 µl, respectively. Resuspended cells were then distributed into 40 µl aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

Agrobacterium cells were transformed with plasmids prepared as described above following the protocol described by Nagel et al. *FEMS Microbiol Letts* 67: 325-328 (1990). For each DNA construct to be transformed, 50-100 ng DNA (generally resuspended in 10 mM Tris-HCI, 1 mM EDTA, pH 8.0) was mixed with 40 µl of *Agrobacterium* cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 µF and 200 µF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2-4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 µg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The integrity of the plasmid construct was verified by PCR amplification and sequence analysis.

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Example V. Transformation of *Arabidopsis* Plants with A*grobacterium tumefaciens* with Expression Vector

After transformation of *Agrobacterium tumefaciens* with plasmid vectors containing the gene, single *Agrobacterium* colonies were identified, propagated, and used to transform *Arabidopsis* plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l spectinomycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance (A_{600}) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044 μ M benzylamino purine (Sigma), 200 μ l/L Silwet L-77 (Lehle Seeds) until an absorbance (A_{600}) of 0.8 was reached.

Prior to transformation, *Arabidopsis thaliana* seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75 μ E/m²/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

The pots were then immersed upside down in the mixture of *Agrobacterium* infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

Example VI. Identification of Arabidopsis Primary Transformants

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H_2O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H_2O . The seeds were stored in the last wash water at 4° C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH

adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75 µE/m²/sec) at 22-23° C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T₁ generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium). Primary transformants are self-crossed and progeny seeds (T2) collected. T2 progeny seeds were germinated on kanamycin as described above and kanamycin resistant seedlings were selected, transferred to soil and analyzed.

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Example VII. Analysis of transgenic Arabidopsis plants

In a first experiment, G157 plants (ie plants expressing the G157 transgene) were grown in 12 hours light. 31 of 40 lines flowered earlier than control plants transformed with a control vector. Mean rosette leaf number of early T1 lines was 12.4+/-0.8 whereas control lines had 27+/-1.2 rosette leaves. 2 of 40 T1 plants flowered at the same time as controls and 7 of 40 lines were late flowering and produced visible inflorescences 2 to 3 weeks after wild type.

In further experiments, plants were grown under conditions of 24 hours light at 20-25 °C. Under these conditions, the non-transformed control plants produced a mean total of 14.3+/-0.7 leaves on the primary shoots prior to flower bud initiation. Flower buds were first visible on these plants at a mean of 21.1+/-0.5 days after sowing (error values represent standard error of the mean to which 95% confidence limits have been attached). For G859, 14/19 T1 plants were early flowering (mean leaf total of 6.4+/-0.7, flower buds visible at 12.9+/-0.7days after sowing), 3/19 were wild type, and 2/19 were slightly late flowering compared to wild type (mean total of 19 leaves, flower buds visible at 27 days). RT expression studies revealed that the late flowering individuals possessed the highest levels of transgene expression. These results strongly parallel those obtained for G157. For G1842, 7/10 T1 flowered early (mean total of 7.9+/-0.6 leaves, flower buds visible at 13.9+/-1.0 days), and 3/10 plants were wild type. Overexpression studies were also performed with cDNAs encoding shortened splice variants of G1842. For G1842.2 (encodes a 185 amino acid splice variant), 15/18 T1 plants flowered early (mean total of 6.9+/-0.9 leaves, flower buds visible at 14.5+/-0.6 days) and 3/18 were wild type. For G1842.6 (encodes a 77 amino acid splice variant), 8/10 T1 plants flowered early mean total of 6.8+/-1.6 leaves, flower buds visible at 13.9+/-0.9 days) and 2/10 were wild type. For G1842.7 (encodes a 118 amino acid splice variant) 8/10 T1 plants flowered early (accurate leaf counts not made) and 2/10 were wild type. Thus, the G1842 splice variants produced comparable effects to the full-length cDNA

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clone when over-expressed. For G1843, 7/11 flowered early (mean total of 6.4+/-0.5 leaves, flower buds visible at 16.0+/-1.6 days) and 2/11 had a wild type flowering time. The G1843 T1 plants, however, were dwarfed and showed retarded development of some organs. This suggests that G1843 has unpredicted toxic effects when over-expressed. For G1844, 6/10 T1 plants flowered early (mean total of 6.8+/-1.7 leaves, flower buds visible at 14.7+/-1.3 days) and 4/10 plants were wild type. Overexpression studies were also performed with a cDNA encoding a shortened splice variant of G1844. For overexpression of G1844.2 (encodes a 184 amino acid splice variant), 6/19 T1 plants flowered early (mean total of 7.8+/-1.7 leaves, flower buds visible at 15.7+/-1.3 days) and 13/19 were wild type). The over-expression data for G859, G1842, G1843, and G1844 support the hypothesis that they have a role in the control of flowering time.

RT-PCR was performed on materials from G157 plants using G157 specific primers at approximately 25 cycles. The highest levels of G157 expression were detected in late flowering individual plants or in samples from pooled seedlings that contained late flowering individuals. Plants that showed only moderate or low levels of overexpression compared to wild type were slightly early flowering or normal.

To test whether an increase in G157 could affect flowering time in late flowering ecotypes of Arabidopsis, we overexpressed G157 in the late flowering ecotypes Stockholm and Piztal. In this experiment, 32 primary transformants from each ecotype were grown interspersed with controls under continuous light conditions. In both ecotypes, around 50% of the transformants flowered earlier than controls, and in some transformants the time to flowering was halved. As was observed with Columbia G157 plants, a minority of Pitztal and Stockholm transformants were clearly later flowering compared to controls.

A correlation between G157 transgene expression and flowering time was also observed in G157 Stockholm and Pitztal T1 plants. RT-PCR was performed with two early and two late flowering lines in each background. Again, the late flowering lines contained the higher levels of G157 expression. Thus, the factor appears to affect flowering time in a quantitative manner; a modest level of overexpression triggers early flowering, whereas a larger increase delays flowering.

In conclusion, over-expression of G157 or any of the related genes modifies flowering time in plants: a modest level of over-expression triggers early flowering, whereas a larger increase delays flowering.

Using similar or identical methodologies described in the examples above, further Arabidopsis genes were identified whose altered expression was correlated with delayed or accelerated flowering. These genes are tabulated in Table 2 with their Sequence Listing Nos., and their effects on flowering time.

Table 2. Further Arabidopsis genes for manipulating flowering time

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SEQ ID Nos.	Gene	observations
23, 24	G861	early or late flowering
25, 26	G861.1	early or late flowering
29, 30	G192	late flowering
31, 32	G234	late flowering
33, 34	G361	late flowering
35, 36	G486	late flowering
37, 38	G748	late flowering
39, 40	G994	late flowering
41, 42	G1335	late flowering
43, 44	G562	late flowering
45, 46	G736	late flowering
47,48	G1073	late flowering
49, 50	G1435	late flowering
51, 52	G180	early flowering
53, 54	G592	early flowering
55, 56	G208	early flowering

The vernalization response was also investigated. Late flowering vernalization responsive ecotypes and mutants have high steady state levels of FLC transcript, which decrease during the promotion of flowering by vernalization (Michaels and Amasino, (1999) Plant Cell 11:949-956; Sheldon et al., (1999) Plant Cell 11:445-458; Sheldon et al., (2000) Proc. Natl. Acad. Sci. 97: 3735-3758). In contrast to FLC, G157 transcript levels show no consistent correlation with the vernalization response in the late flowering Stockholm and Pitztal ecotypes. Additionally we found that over-expression of G157 did not influence FLC levels. The effects of vernalization on expression of G861, G859, G1842, G1843, and G1844 were also examined. Germinating seeds of Columbia, Pitztal, Stockholm, constans-1, and fca-9 were vernalized on MS agar plates in a 4°C cold room for 8 weeks, and then transferred to a continuous light growth room. Total tissues from the vernalized seedlings, and freshly sown non-vernalized controls were harvested at 9 days after the transfer. RT-PCR was performed for FLC, G157, G859, G1842, G1843, G1844, and G861, and actin. Compared to FLC and G157, none of the genes showed a clear consistent decline upon vernalization in the five different sample sets. However, G1844 displayed a converse pattern of expression to FLC: G1844 levels consistently increased on vernalization. This is particularly significant as it directly implicates G1844 in control of the vernalization response. Thus G1844 likely activates

flowering and has an opposing role to FLC.

To explore whether overexpression of G157 produces comparable effects on vernalization, batches of wild type Pitztal and Stockholm seedlings were cold treated for 6 weeks at 4°C, then grown amongst a second selection of G157 T1 Pitztal, G157 T1 Stockholm and non-vernalized wild type plants. As expected, vernalization markedly and uniformly reduced flowering time in both Pitztal and Stockholm wild type plants. Amongst the G157 Stockholm lines, the earliest flowering T1 group (8/23 lines) was indistinguishable from vernalized plants. For Pitztal, however, the early flowering T1 plants were on average marginally later than the vernalized plants. Therefore, overexpression of G157 can substantially reduce the requirement for vernalization in late flowering ecotypes.

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Additionally, we observed that the late flowering of G157 lines is independent of FLC expression and does not respond to vernalization. However, the late flowering G157 plants are responsive to photoperiod. In an experiment conducted under short day conditions of 8 hours of light, we obtained a number of G157 Columbia T1 plants that flowered up to a month later than wild type controls (data not shown). To confirm that the late flowering effects caused by G157 overexpression were independent of *FLC* transcription, we tested whether late flowering G157 Columbia plants were responsive to vernalization. No significant change in flowering time was noted: in continuous light conditions, vernalized T2 plants of line 4 had a total of 31.3 +/- 1.8 leaves compared to 30.1 +/- 1.3 when non-vernalized. Control *fca* plants verified that the treatment was effective: vernalized plants flowered after only 10.3 +/- 0.9 leaves compared to more than 40 leaves for the non-vernalized controls. Thus, the late flowering phenotype caused by G157 could not be overcome by vernalization, as would be expected if the delay occurred independently of changes in *FLC* expression

Example IX. Identification of Homologous Sequences

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Homologs from plant species other than Arabidopsis were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410; and Altschul et al. (1997) *Nucl. Acid Res.* 25: 3389-3402). The tblastx sequence analysis programs were employed using the BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919).

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The entire NCBI Genbank database was filtered for sequences from all plants except Arabidopsis thaliana by selecting all entries in the NCBI Genbank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (Arabidopsis thaliana). These sequences were compared to sequences representing genes of SEQ IDs 1-56 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs 1-56, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of 3.6e-40 is 3.6 x 10⁻⁴⁰. For up to ten

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species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 2.

In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-Arabidopsis genes shown in Figure 2 and the Arabidopsis genes in the sequence listing are: SEQ ID No. 1: 54%-67%; SEQ ID Nos. 3,5,7: 37%-47%; SEQ ID Nos. 9,11,13,15: 54%-62%; SEQ ID No. 17: 62%-71%; SEQ ID Nos. 19, 21: 50%-67%; SEQ ID Nos. 23,25: 75%-91%; SEQ ID No. 27: 46%-69%; SEQ ID No. 29: 44%-90%; SEQ ID No. 31: 57-89%; SEQ ID No. 33: 37%-79%; SEQ ID No. 35: 50%-71%; SEQ ID No. 37: 39%-63%; SEQ ID No. 39: 58%-70%; SEQ ID No. 41: 45%-73%; SEQ ID No. 43: 42%-84%; SEQ ID No. 45: 47%-81%; SEQ ID No. 47: 31%-71%; SEQ ID No. 49: 40%-67%; SEQ ID No. 51: 69%-51%; SEQ ID No. 53: 43%-86%; and SEQ ID No. 55: 79%-89%.

Arabidopsis homologs of genes in Table 2 were also identified using BLAST. These genes are found in the following Arabidopsis BAC sequences, identified by their Genbank sequence NID numbers: 2827698 (G234 homolog), 3241917 (G748 homolog), 2618604 (G994 homolog), 6598548 (G1335 homolog), 7340331 (G736 homolog), 6523051 (G1435 homolog), 6598491 (G208 homolog) and 3172156 (G208 homolog).

All references (publications and patents) are incorporated herein by reference in their entirety for all purposes.

Although the invention has been described with reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

Figure 1

SEQ ID No.	Gene	cDNA or	conserved doman
1	G157	cDNA	
2	G157	protein	2-57
3	G859	cDNA	
4	G859	protein	2-57
5	G859.1	cDNA	
6	G859.1	protein	2-57
7	G859.2	cDNA	
8	G859.2	protein	2-57
9	G1842	cDNA	
10	G1842	protein	2-57
11	G1842.2	cDNA	
12	G1842.2	protein	2-57
13	G1842.6	cDNA	
14	G1842.6	protein	2-57
15	G1842.7	cDNA	
16	G1842.7	protein	2-57
17	G1843	cDNA	2-01
18	G1843	protein	2-57
19	G1844	cDNA	2-01
20			2-57
	G1844	protein	2-57
21	G1844.2	cDNA	
22	G1844.2	protein	2-57
23	G861	cDNA	<u> </u>
24	G861	protein	2-57
25	G861.1	cDNA	
26	G861.1	protein	2-57
27	G1759	cDNA	
28	G1759	protein	2-57
29	G192	cDNA	
30	G192_	protein	128-185
31	G234	cDNA	
32	G234	protein	14-115
33	G361	cDNA	
34	G361_	protein	43-63
35	G486	cDNA	
36	G486	protein	5-66
37	G748	cDNA	
38	G748	protein	112-140
39	G994	cDNA	
40	G994	protein	14-123
41	G1335	cDNA	
42	G1335	protein	24-43, 131-144, 185-203
43	G562	cDNA	
44	G562	protein	253-315
45	G736	cDNA	
46	G736	protein	54-111
47	G1073	cDNA	
48	G1073	protein	33-42, 78-175
49	G1435	cDNA	
50	G1435	protein	146-194
51	G180	cDNA	
52	G180		118-174
53	G592	protein cDNA	110-174
			290-342
54	G592	protein ·	290-342
55	G208	cDNA	144 446
56	G208	protein	14-116

Figure 2A

Figure ZA				,
SEQ IDs	Gene Ids	Genbank NID	P-value	Species
1	G157	6530836	3.10E-22	Lycopersicon esculentum
1	G157	5606765	5.50E-14	Glycine max
1	G157	6826955	1.20E-13	Zea mays
1	G157	6536942	6.00E-13	Medicago truncatula
1	G157	8707754	1.40E-12	Hordeum vulgare
1	G157	2293891	1.40E-12	Petunia x hybrida
1	G157	19870	1.40E-12	Nicotiana tabacum
1	G157	7628118	3.70E-12	Gossypium arboreum
1	G157	5050220	3.80E-12	Gossypium hirsutum
1	G157	9414215	4.50E-12	Triticum aestivum
3,5,7	G859	6530836	1.40E-34	Lycopersicon esculentum
3,5,7	G859	5777903	4.70E-30	Malus domestica
3,5,7	G859	9367312	7.10E-30	Hordeum vulgare
3,5,7	G859	6467973	3.60E-29	Dendrobium grex Madame Thong-IN
3,5,7	G859	4204233	1.20E-28	Lolium temulentum
3,5,7	G859	939784	2.50E-28	Zea mays
3,5,7	G859	6651032	3.10E-28	Capsicum annuum
3,5,7	G859	1483227	4.60E-28	Betula pendula
3,5,7	G859	5295983	8.70E-28	Oryza sativa
3,5,7	G859	5070137	1.10E-27	Nicotiana sylvestris
9,11,13,15	G1842	6530836	5.90E-19	Lycopersicon esculentum
9,11,13,15	G1842	5606765	8.00E-15	Glycine max
9,11,13,15	G1842	6826955	1.20E-12	Zea mays
9,11,13,15	G1842	4979250	1.50E-11	Oryza sativa
9,11,13,15	G1842	6536942	1.50E-11	Medicago truncatula
9,11,13,15	G1842	7501504	4.00E-11	Gossypium arboreum
9,11,13,15	G1842	9444818	4.70E-11	Triticum aestivum
9,11,13,15	G1842	5859176	5.40E-11	Pinus taeda
9,11,13,15	G1842	5777905	6.80E-11	Malus domestica
9,11,13,15	G1842	6647105	6.80E-11	Mesembryanthemum crystallinum
17	G1843	8707754	6.60E-15	Hordeum vulgare
17	G1843	5606765	1.10E-14	Glycine max
17	G1843	4387730	1.50E-14	Lycopersicon esculentum
17	G1843	3646325	1.60E-14	Malus domestica
17	G1843	7625048	1.60E-14	Gossypium arboreum
17	G1843	5050220	1.80E-14	Gossypium hirsutum
17	G1843	9429009	3.00E-14	Triticum aestivum
17	G1843	7145381	6.30E-14	Zea mays
17	G1843	3824730	8.20E-14	Oryza sativa
17	G1843	4528048	2.30E-13	Citrus unshiu
19, 21	G1844	5606765	1.70E-14	Glycine max
19, 21	G1844	8707754	3.30E-13	Hordeum vulgare
19, 21	G1844	9429009	4.40E-13	Triticum aestivum
19, 21	G1844	4979250	1.00E-12	Oryza sativa
19, 21	G1844	7628118	1.10E-12	Gossypium arboreum
19, 21	G1844	5050220	1.20E-12	Gossypium hirsutum
19, 21	G1844	6530836	1.40E-12	Lycopersicon esculentum
19, 21	G1844	6918768	1.70E-12	Zea mays
19, 21	G1844	6536942	3.50E-12	Medicago truncatula
19, 21	G1844	2252481	3.70E-12	Ceratopteris richardii
23,25	G861	5601313	8.20E-49	Lycopersicon esculentum
23,25	G861	2735763	1.50E-37	Solanum tuberosum
	G861	6652755	5.40E-37	Paulownia kawakamii
23,25	10001	0002700	0.40⊏-37	r autowilla nawanai!!!!

Figure 2B

Figure 2B				
SEQ IDs	Gene Ids	Genbank NID	P-value	Species
23,25	G861	9367233	3.30E-33	Hordeum vulgare
23,25	G861	7672990	8.90E-29	Canavalia lineata
23,25	G861	3986688	5.20E-26	Cichorium intybus
23,25	G861	7552197	2.40E-25	Sorghum bicolor
23,25	G861	5295977	4.90E-24	Oryza sativa
23,25	G861	9194959	3.60E-19	Medicago truncatula
23,25	G861	3855425	4.40E-19	Populus tremula x Populus tremuloides
27	G1759	5606765	4.60E-16	Glycine max
27	G1759	7647685	4.10E-15	Lycopersicon esculentum
27	G1759	4979250	2.70E-14	Oryza sativa
27	G1759	8707754	6.30E-14	Hordeum vulgare
27	G1759	5777905	6.80E-14	Malus domestica
27	G1759	7626240	1.10E-13	Gossypium arboreum
27	G1759	5047371	1.10E-13	Gossypium hirsutum
27	G1759	6918768	1.20E-13	Zea mays
27	G1759	8574456	1.30E-13	Capsicum annuum
27	G1759	8216956	1.30E-13	Cucumis sativus
29	G192	7284340	3.60E-40	Glycine max
29	G192	7779802	1.10E-39	Lotus japonicus
29	G192	9361307	9.40E-28	Triticum aestivum
29	G192	7340336	8.10E-24	Oryza sativa
29	G192	6529152	4.70E-23	Lycopersicon esculentum
29	G192	7206269	2.90E-22	Medicago truncatula
29	G192	4886128	4.50E-15	Zea mays
29	G192	8706346	4.70E-13	Hordeum vulgare
29	G192	9302479	8.80E-13	Sorghum bicolor
29	G192	3326241	2.40E-12	Gossypium hirsutum
31	G234	9193243	7.50E-60	Medicago truncatula
31	G234	9264511	3.30E-57	Glycine max
31	G234	7412424	3.60E-49	Lycopersicon esculentum
31	G234	8335078	2.60E-48	Oryza sativa
31	G234	7218651	1.00E-42	Sorghum bicolor
31	G234	9364630	9.90E-40	Triticum aestivum
31	G234	6079814	5.10E-36	Gossypium arboreum
31	G234	9252441	5.40E-35	Solanum tuberosum
31	G234	5860031	1.00E-33	Pinus taeda
31	G234	5050757	2.60E-33	Gossypium hirsutum
33	G361	7561045	2.30E-21	Medicago truncatula
33	G361	9307604	1.20E-17	Sorghum bicolor
33	G361	4119050	1.70E-13	Oryza sativa
33	G361	8175037	7.30E-13	Hordeum vulgare
33	G361	8329902	5.30E-09	Mesembryanthemum crystallinum
33	G361	6534259	1.20E-08	Lycopersicon esculentum
33	G361	7283798	1.30E-08	Glycine max
33	G361	3854369	5.50E-08	Populus tremula x Populus tremuloides
33	G361	9365078	1.70E-07	Triticum aestivum
33	G361	5268965	0.00023	Zea mays
35	G486		3.10E-36	Glycine max
35		8172030	4.20E-29	Medicago truncatula
35	G486	9416562	6.40E-29	Triticum aestivum
35	G486		4.90E-28	Gossypium hirsutum
35 35		7628400	6.10E-28	Gossypium arboreum
	G486			
35	G486	7781090	2.10E-27	Lotus japonicus

Figure 2C

35 G486 22379 3.40E-27 Zea mays 35 G486 9441376 4.80E-27 Chlamydomonas reinhardtii 35 G486 7409616 1.30E-26 Lycopersicon esculentum 35 G486 8071558 1.40E-26 Solanum tuberosum 37 G748 853689 5.60E-87 Cucurbita maxima 37 G748 583689 5.60E-87 Cucurbita maxima 37 G748 588560 9.70E-46 Lycopersicon esculentum 37 G748 6341666 4.50E-38 Glycine max 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 9191944 1.70E-31 Hordeum vulgare 37 G748 9419494 1.70E-31 Hordeum vulgare 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 6029953 7.30E-21 Zea mays<	Figure 2C		1		1
35 G486 9441376 4.80E-27 Chlamydomonas reinhardtii 35 G486 7409616 1.30E-26 Lycopersicon esculentum 35 G488 8071558 1.40E-26 Solanum tuberosum 37 G748 853689 5.60E-87 Cucurbita maxima 37 G748 5888560 9.70E-46 Lycopersicon esculentum 37 G748 6341666 4.50E-38 Glycine max 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 9190140 2.90E-31 Hordeum vulgare 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 9420953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago	SEQ IDs	Gene Ids	Genbank NID	P-value	Species
35 G486 7409616 1.30E-26 Lycopersicon esculentum 35 G486 8071558 1.40E-26 Solanum tuberosum 37 G748 853689 5.60E-87 Cucurbita maxima 37 G748 5888560 9.70E-46 Lycopersicon esculentum 37 G748 6341666 4.50E-38 Glycine max 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 9419494 1.70E-31 Hordeum vulgare 37 G748 9419494 1.70E-31 Hordeum vulgare 37 G748 9419494 1.70E-31 Hordeum vulgare 37 G748 941957 8.20E-29 Triticum aestivum 37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 6020953 7.30E-21 Zea mays 39 G994 7661750 5.60E-51 Medicago truncatula 39 G994 7561750 5.60E-51 Medicago trun	35	G486	22379	3.40E-27	Zea mays
35 G486 8071558 1.40E-26 Solanum tuberosum 37 G748 853689 5.60E-87 Cucurbita maxima 37 G748 5888560 9.70E-46 Lycopersicon esculentum 37 G748 6341666 4.50E-38 Glycine max 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 7535776 4.00E-33 Sorghum bicolor 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 99293224 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 8020953 7.30E-21 Zea mays 39 G994 7651750 5.60E-51 Medicago truncatula 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 19452884 3.10E-50 Lycopersicon escu	35	G486	9441376	4.80E-27	Chlamydomonas reinhardtii
37 G748 853689 5.60E-87 Cucurbita maxima 37 G748 7242897 3.10E-59 Oryza sativa 37 G748 5888560 9.70E-46 Lycopersicon esculentum 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 9419494 1.70E-31 Medicago truncatula 37 G748 9419194 1.70E-31 Hordeum vulgare 37 G748 9410197 8.20E-29 Triticum aestivum 37 G748 9410197 8.20E-29 Triticum aestivum 37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 6020953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 1430845 3.10E-50 Zea mays 39 G994 14561750 5.60E-51 Medicago tr	35	G486	7409616	1.30E-26	Lycopersicon esculentum
37 G748 7242897 3.10E-59 Oryza sativa 37 G748 6841666 4.50E-38 Glycine max 37 G748 6341666 4.50E-38 Glycine max 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 7535776 4.00E-33 Sorghum bicolor 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 6020953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 125282 5.40E-49 Oryza sativa 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 726566 4.40E-44 Gossypium arboreum	35	G486	8071558	1.40E-26	Solanum tuberosum
37 G748 7242897 3.10E-59 Oryza sativa 37 G748 68341666 4.50E-38 Glycine max 37 G748 6341666 4.50E-38 Glycine max 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 7535776 4.00E-33 Sorghum bicolor 37 G748 9410157 6.20E-29 Triticum aestivum 37 G748 9410157 6.20E-29 Triticum aestivum 37 G748 9410157 6.20E-29 Triticum aestivum 37 G748 942924 3.50E-25 Dendrobium grex Madame Thong-IN 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 1945282 5.40E-49 Oryza sativa	37	G748	853689	5.60E-87	Cucurbita maxima
37 G748 6341666 4.50E-38 Glycine max 37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 7535776 4.00E-33 Sorghum bicolor 37 G748 9419494 1.70E-31 Hordeum vulgare 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 6020953 7.30E-21 Zea mays 39 G994 6661291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 7526566 4.40E-44 Gossypium hirsutum 39 G994 72921339 4.50E-44 Gossypium hirsutu	37		7242897	3.10E-59	Oryza sativa
37 G748 9190140 2.90E-35 Medicago truncatula 37 G748 7535776 4.00E-33 Sorghum bicolor 37 G748 9419494 1.70E-31 Hordeum vulgare 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 6020953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 5268844 2.10E-50 Zea mays 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1495282 5.40E-49 Oryza sativa 39 G994 7263666 4.40E-44 Gossypium arboreum 39 G994 7262666 4.40E-44 Gossypium arboreum 39 G994 7290249 3.60E-43 Petunia x hybrida 41 G1335 159742 8.40E-63 Nicotiana sylvestris	37	G748	5888560	9.70E-46	Lycopersicon esculentum
37 G748 7535776 4.00E-33 Sorghum bicolor 37 G748 9419494 1.70E-31 Hordeum vulgare 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 6020953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 156844 2.10E-50 Zea mays 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1495282 5.40E-49 Oryza sativa 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 7626566 4.40E-44 Gossypium arboreum 39 G994 7690249 3.60E-43 Glycine max 39 G994 7590249 3.60E-43 Glycine max	37	G748	6341666	4.50E-38	Glycine max
37 G748 9419494 1.70E-31 Hordeum vulgare 37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 6020953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 5268844 2.10E-50 Zea mays 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 7626566 4.40E-44 Gossyplum abroreum 39 G994 7590249 3.60E-43 Glycine max 39 G994 7590249 3.60E-43 Petunia x hybrida 41 G1335 5398738 1.20E-59 Zea mays	37	G748	9190140	2.90E-35	Medicago truncatula
37 G748 9410157 8.20E-29 Triticum aestivum 37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 145282 5.40E-49 Oryza sativa 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 22637 1.40E-46 Physcomitrella patens 39 G994 7626566 4.40E-44 Gossypium arboreum 39 G994 7590249 3.60E-43 Glycine max 39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Glycine max 41 G1335 5398738 1.20E-59 Zea mays	37	G748	7535776	4.00E-33	Sorghum bicolor
37 G748 3929324 3.50E-25 Dendrobium grex Madame Thong-IN 37 G748 6020953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 5268844 2.10E-50 Zea mays 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 266566 4.40E-44 Gossypium arboreum 39 G994 2921339 4.50E-44 Gossypium hirsutum 39 G994 20562 6.30E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 5398738 1.20E-59 Zea mays	37	G748	9419494	1.70E-31	Hordeum vulgare
37 G748 6020953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 5268844 2.10E-50 Zea mays 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 22637 1.40E-46 Physcomitrella patens 39 G994 7626566 4.40E-44 Gossypium hirsutum 39 G994 7626566 4.40E-44 Gossypium hirsutum 39 G994 7590249 3.60E-43 Glycine max 39 G994 7590249 3.60E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum	37	G748	9410157	8.20E-29	Triticum aestivum
37 G748 6020953 7.30E-21 Zea mays 39 G994 6651291 1.50E-55 Pimpinella brachycarpa 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 5268844 2.10E-50 Zea mays 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 22637 1.40E-46 Physcomitrella patens 39 G994 7626566 4.40E-44 Gossypium arboreum 39 G994 7626566 4.40E-44 Gossypium brisutum 39 G994 7590249 3.60E-43 Glycine max 39 G994 7590249 3.60E-43 Retunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum		G748		3.50E-25	Dendrobium grex Madame Thong-IN
39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 5268844 2.10E-50 Zea mays 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 22637 1.40E-46 Physcomitrella patens 39 G994 7626566 4.40E-44 Gossypium arboreum 39 G994 2951339 4.50E-44 Gossypium hirsutum 39 G994 29562 6.30E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare	37	G748	6020953	7.30E-21	Zea mays
39 G994 7561750 5.60E-51 Medicago truncatula 39 G994 5268844 2.10E-50 Zea mays 39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 12637 1.40E-46 Physcomitrella patens 39 G994 22637 1.40E-44 Gossypium arboreum 39 G994 7626566 4.40E-44 Gossypium hirsutum 39 G994 7590249 3.60E-43 Glycine max 39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Glycine max 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 830366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare <	39	G994	6651291		Pimpinella brachycarpa
39 G994 1430845 3.10E-50 Lycopersicon esculentum 39 G994 1945282 5.40E-49 Oryza sativa 39 G994 22637 1.40E-46 Physcomitrella patens 39 G994 7626566 4.40E-44 Gossypium arboreum 39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 77502173 2.60E-37 Gossypium arboreum 41 G1335 7550978 3.30E-35 Sorghum bicolor		G994	7561750	5.60E-51	
39 G994 1945282 5.40E-49 Oryza sativa 39 G994 22637 1.40E-46 Physcomitrella patens 39 G994 7626566 4.40E-44 Gossypium arboreum 39 G994 2921339 4.50E-44 Gossypium hirsutum 39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 87721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 7550978 3.30E-35 Sorghum bicolor <td>39</td> <td>G994</td> <td>5268844</td> <td>2.10E-50</td> <td>Zea mays</td>	39	G994	5268844	2.10E-50	Zea mays
39 G994 1945282 5.40E-49 Oryza sativa 39 G994 22637 1.40E-46 Physcomitrella patens 39 G994 7626566 4.40E-44 Gossypium arboreum 39 G994 2921339 4.50E-44 Gossypium hirsutum 39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum <td>39</td> <td>G994</td> <td>1430845</td> <td>3.10E-50</td> <td>Lycopersicon esculentum</td>	39	G994	1430845	3.10E-50	Lycopersicon esculentum
39 G994 22637 1.40E-46 Physcomitrella patens 39 G994 7626566 4.40E-44 Gossypium arboreum 39 G994 2921339 4.50E-44 Gossypium hirsutum 39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 7550978 3.30E-35 Sorghum bicolor	39	G994	1945282	5.40E-49	
39 G994 2921339 4.50E-44 Gossypium hirsutum 39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 6696628 8.00E-42 Pinus taeda 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus	39	G994	22637		Physcomitrella patens
39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 7696628 8.00E-42 Pinus taeda 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus	39	G994	7626566	4.40E-44	Gossypium arboreum
39 G994 7590249 3.60E-43 Glycine max 39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 1817176 5.60E-36 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus	39	G994	2921339		Gossypium hirsutum
39 G994 20562 6.30E-43 Petunia x hybrida 41 G1335 19742 8.40E-63 Nicotiana sylvestris 41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 6696628 8.00E-42 Pinus taeda 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max	39	G994	7590249	3.60E-43	
41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 6696628 8.00E-42 Pinus taeda 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 1817176 5.60E-36 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 1399004 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 728627 4.50E-41 Nicotiana tabacum <td>39</td> <td>G994</td> <td>20562</td> <td>6.30E-43</td> <td></td>	39	G994	20562	6.30E-43	
41 G1335 5398738 1.20E-59 Zea mays 41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 6696628 8.00E-42 Pinus taeda 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 1817176 5.60E-36 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum <	41	G1335	19742	8.40E-63	Nicotiana sylvestris
41 G1335 9361467 1.40E-50 Triticum aestivum 41 G1335 8330366 1.60E-48 Mesembryanthemum crystallinum 41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 6696628 8.00E-42 Pinus taeda 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 1817176 5.60E-36 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum <td>41</td> <td>G1335</td> <td>5398738</td> <td>1.20E-59</td> <td></td>	41	G1335	5398738	1.20E-59	
41 G1335 8174823 7.50E-43 Hordeum vulgare 41 G1335 6696628 8.00E-42 Pinus taeda 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 7550978 3.30E-35 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum <td>41</td> <td>G1335</td> <td>9361467</td> <td>1.40E-50</td> <td></td>	41	G1335	9361467	1.40E-50	
41 G1335 6696628 8.00E-42 Pinus taeda 41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 1817176 5.60E-36 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crys	41	G1335	8330366	1.60E-48	Mesembryanthemum crystallinum
41 G1335 7721100 1.20E-39 Lotus japonicus 41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 1817176 5.60E-36 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crysta	41	G1335	8174823	7.50E-43	Hordeum vulgare
41 G1335 7502173 2.60E-37 Gossypium arboreum 41 G1335 1817176 5.60E-36 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	41	G1335	6696628	8.00E-42	Pinus taeda
41 G1335 1817176 5.60E-36 Pinus radiata 41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 832888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	41	G1335	7721100	1.20E-39	Lotus japonicus
41 G1335 7550978 3.30E-35 Sorghum bicolor 43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 832888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	41	G1335	7502173	2.60E-37	Gossypium arboreum
43 G562 1399004 6.60E-142 Brassica napus 43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 832888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	41	G1335	1817176	5.60E-36	Pinus radiata
43 G562 5381310 6.80E-53 Catharanthus roseus 43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 832888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	41	G1335	7550978	3.30E-35	Sorghum bicolor
43 G562 169958 3.80E-45 Glycine max 43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 832888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	43	G562	1399004	6.60E-142	Brassica napus
43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	43	G562	5381310	6.80E-53	Catharanthus roseus
43 G562 2879779 3.60E-43 Spinacia oleracea 43 G562 7565950 2.10E-41 Medicago truncatula 43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	43	G562	169958	3.80E-45	Glycine max
43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	43			3.60E-43	
43 G562 728627 4.50E-41 Nicotiana tabacum 43 G562 1155053 2.30E-40 Phaseolus vulgaris 43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum		G562	7565950	2.10E-41	
43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	43	G562	728627	4.50E-41	Nicotiana tabacum
43 G562 1498300 5.70E-40 Petroselinum crispum 43 G562 5046889 6.70E-34 Gossypium hirsutum 43 G562 8328888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum			1155053	2.30E-40	Phaseolus vulgaris
43 G562 8328888 2.60E-25 Mesembryanthemum crystallinum 45 G736 7409627 1.40E-37 Lycopersicon esculentum	43	G562	1498300	5.70E-40	Petroselinum crispum
45 G736 7409627 1.40E-37 Lycopersicon esculentum	43	G562	5046889	6.70E-34	Gossypium hirsutum
	43	G562	8328888	2.60E-25	Mesembryanthemum crystallinum
45 G736 Q197391 5.60E-32 Medicago truncatula	45	G736	7409627	1.40E-37	Lycopersicon esculentum
45 0756 9197591 0.00E-52 Medicago trancatala	45	G736	9197391	5.60E-32	Medicago truncatula
45 G736 9419494 4.70E-27 Hordeum vulgare	45		9419494		
45 G736 7328718 1.30E-25 Oryza sativa					
45 G736 9410157 1.80E-25 Triticum aestivum					Triticum aestivum
45 G736 853689 5.20E-25 Cucurbita maxima					Cucurbita maxima
45 G736 7535776 6.60E-25 Sorghum bicolor					
45 G736 3929324 4.70E-21 Dendrobium grex Madame Thong-IN					
45 G736 2393774 9.60E-20 Zea mays					

Figure 2D

I igule 2D	I _	T	<u></u>	T
SEQ IDs	Gene Ids	Genbank NID	P-value	Species
45	G736	7624398	1.10E-19	Gossypium arboreum
47	G1073	7718401	2.20E-55	Medicago truncatula
47	G1073	6846994	2.50E-44	Glycine max
47	G1073	7615218	1.60E-42	Lotus japonicus
47	G1073	7333102	2.70E-34	Lycopersicon esculentum
47	G1073	9445090	3.40E-25	Triticum aestivum
47	G1073	9252370	2.20E-24	Solanum tuberosum
47	G1073	5042437	4.60E-21	Oryza sativa
47	G1073	7536402	5.30E-20	Sorghum bicolor
47	G1073	2213535	7.30E-19	Pisum sativum
47	G1073	7624850	2.10E-18	Gossypium arboreum
49	G1435	9203811	3.70E-37	Glycine max
49	G1435	9430136	4.10E-35	Lycopersicon esculentum
49	G1435	8904354	4.30E-32	Hordeum vulgare
49	G1435	5050706	3.30E-26	Gossypium hirsutum
49	G1435	7614196	6.40E-19	Lotus japonicus
49	G1435	7551484	1.00E-18	Sorghum bicolor
49	G1435	6916552	7.20E-12	Lycopersicon pennellii
49	G1435	2443007	5.50E-11	Oryza sativa
49	G1435	9255229	1.30E-10	Zea mays
49	G1435	7766737	2.80E-10	Medicago truncatula
51	G180	8468047	1.90E-35	Oryza sativa
51	G180	7559831	1.20E-24	Medicago truncatula
51	G180	5272716	9.90E-24	Lycopersicon esculentum
51	G180	9187621	3.30E-23	Solanum tuberosum
51	G180	6566312	1.30E-22	Glycine max
51	G180	9304207	1.30E-21	Sorghum bicolor
51	G180	7721184	1.30E-20	Lotus japonicus
51	G180	9444636	3.10E-19	Triticum aestivum
51	G180	3220212	5.20E-19	Gossypium hirsutum
51	G180	1159876	8.00E-19	Avena fatua
53	G592	7924069	7.10E-27	Glycine max
53	G592	5896650	1.10E-22	Lycopersicon esculentum
53	G592	6279773	1.10E-17	Lycopersicon pennellii
53	G592	9364330	1.20E-14	Triticum aestivum
53	G592	6166282	5.40E-14	Pinus taeda
53	G592	8367093	1.60E-12	Zea mays
53	G592	9301543	6.60E-11	Sorghum bicolor
53	G592	7562632	2.80E-10	Medicago truncatula
53	G592	702652	5.80E-05	Oryza sativa
53	G592	7322923	0.094	Lycopersicon hirsutum
55	G208	437326	2.80E-65	Gossypium hirsutum
55	G208	7765706	4.40E-64	Medicago truncatula
55	G208	5269878	5.80E-64	Lycopersicon esculentum
55 55	G208	19054	6.90E-63	Hordeum vulgare
55	G208	2605616	1.00E-62	Oryza sativa
55 55	G208	7626566	3.50E-62	Gossypium arboreum
55 55		6667606	4.10E-62	Glycine max
55	G208	517492		
	G208		1.80E-60	Zea mays
55 55	G208	9302672	2.40E-57	Sorghum bicolor
55	G208	5860031	1.30E-54	Pinus taeda

We Claim:

1. A transgenic plant comprising a recombinant polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising at least 6 consecutive amino acids of a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28 but excluding SEQ ID No. 28, wherein said transgenic plant has (i) a modified flowering time compared with another plant lacking the recombinant polynucleotide or (ii) a modified vernalization requirement compared with another plant lacking the recombinant polynucleotide.

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- 2. The transgenic plant of claim 1, wherein the nucleotide sequence encodes a polypeptide comprising a conserved domain selected from the group consisting of conserved domains of SEQ ID Nos. 2N, where N=1-28.
- 15 3. The transgenic plant of claim 1, wherein the recombinant polynucleotide further comprises a promoter operably linked to said nucleotide sequence.
 - 4. The transgenic plant of claim 3, wherein said promoter is constitutive or inducible or tissueactive.

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5. The transgenic plant of claim 1, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.

25 6. A method for altering the flowering time or vernalization requirement of a plant, said method comprising (a) transforming a plant with a recombinant polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising at least 6 consecutive amino acids of a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28 but excluding SEQ ID No. 28, (b) selecting said transformed plants; and (c) identifying a transformed plant having an altered flowering time. 30

The method of claim 6, wherein the nucleotide sequence encodes a polypeptide comprising a conserved domain selected from the group consisting of conserved domains of SEQ ID Nos. 2N, where N=1-28.

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8. The method of claim 6, wherein the recombinant polynucleotide further comprises a promoter operably linked to said nucleotide sequence.

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9. The method of claim 8, wherein said promoter is constitutive or inducible or tissue-active.

10. The method of claim 1, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.

- 11. A method for altering the flowering time or vernalization requirement of a plant, said method comprising (a) transforming the plant with a recombinant polynucleotide comprising a nucleotide sequence comprising at least 18 consecutive nucleotides of a sequence selected from the group consisting of SEQ ID Nos. 2N-1, where N= 1-28, but excluding SEQ ID No. 27; and (b) selecting said transformed plant.
- 12. The method of claim 11, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.
- 13. A method for altering a plant's flowering time or vernalization requirement, said method comprising (a) providing a database sequence; (b) comparing said database sequence with a polypeptide selected from SEQ ID Nos. 2N, where N= 1-28; (c) selecting a database sequence that meets selected sequence criteria; and (d) transforming said selected database sequence in the plant.
- 14. The method of claim 13, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.
- 15. A method for altering a plant's flowering time or vernalization requirement, said method comprising (a) providing a database sequence; (b) comparing said database sequence with a polynucleotide selected from SEQ ID Nos. 2N-1, where N= 1-28; (c) selecting a database sequence that meets selected sequence criteria; and (d) transforming said selected database sequence in the plant.
- 16. The method of claim 15, wherein said recombinant polynucleotide encodes a polypeptide comprising a conserved domain having greater than an 84% sequence identity to a sequence selected from the group consisting of SEQ ID Nos. 2N, where N=1-28.

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Page 15

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MBI-0021.txt

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Page 30

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Page 32

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MBI-0021.txt

Page 35

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Gly Ser Gly Ser 35	Glu Ser Arg Lys Tyr 40	Glu Cys Gln Tyr 45	Cys Cys Arg
Glu Phe Ala Asn 50	Ser Gln Ala Leu Gly 55	Gly His Gln Asn 60	Ala His Lys
Lys Glu Arg Gln 65	Leu Leu Lys Arg Ala 70	Gln Met Leu Ala 75	Thr Arg Gly 80
Leu Pro Arg His	His Asn Phe His Pro 85	His Thr Asn Pro	Leu Leu Ser 95
Ala Phe Ala Pro	Leu Pro His Leu Leu 105	Ser Gln Pro His	Pro Pro Pro 110

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His Gly Gly	Ser Leu 165		Met Arg	Arg Phe 170	Ala Gly		er Asp 75	
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PCT/US00/28141 WO 01/26459

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Glu Ala Ser Glu Lys Cys His Arg Glu Asn Arg Lys Thr Val Asn Gly

Asp Asp Ile Trp Trp Ala Leu Ser Thr Leu Gly Leu Asp Asn Tyr Ala

Asp Ala Val Gly Arg His Leu His Lys Tyr Arg Glu Ala Glu Arg Glu

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ctt gad Leu Asj															691
gaa gc Glu Ala 20	Gln														739
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		aga Arg 265														931
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		tac Tyr														1027
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PCT/US00/28141 WO 01/26459

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Pro 305	Pro	Gly	Phe	Tyr	Pro 310	Pro	Pro	Gly	Tyr	Pro 315	Met	Pro	Phe	Tyr	Pro 320
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Trp Ser Lys Ile Ala Val Glu Leu Pro Gly Arg Thr Asp Asn Asp Ile

Lys Asn Tyr Trp Asn Thr His Ile Lys Arg Lys Leu Ile Arg Met Gly

PCT/US00/28141 WO 01/26459

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Val Ser Val Ala Leu Lys Asn Asp Thr 165	Ser Ala Val Leu Ser Gly Asn 170 175
Leu Asn Gln Leu Ala Asp Val Asp Gly 180 185	
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WO 01/26459	PCT/US00/28141
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MBI-0021.txt

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Ala Arg Arg Ser Arg Leu Arg Lys Gln Ala Glu Thr Glu Glu Leu Ala 275 280 285

Arg Lys Val Glu Ala Leu Thr Ala Glu Asn Met Ala Leu Arg Ser Glu 290 295 300

Page 51

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Gln Val Glu L 130	eu Ile Asn Gly 135	Leu Leu Val	Glu Glu Trp Gln 140	His Ala
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Ser Glu Val Thr Gly Ser Gly Ala Gln Ala Cys Glu Ser Asn Leu Gln

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97

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					gca Ala											385
					gaa Glu											433
					ccg Pro											481
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Ile His Asp Val Asn Arg Ala Ser Gln Ile Thr Leu Ser Ser Leu Arg 65 70 75 80

Ser Ser Thr Asn Ala Ser Ser Val Met Glu Glu Val Val Asp Arg Val 85 90 95

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Gly Glu Ala Ala Ala Val Glu Asp Ser Thr Ala Glu Glu Gly Asp $115 \\ 120 \\ 125$

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Val Trp Thr Pro Gln Leu His Lys Arg Phe Val Asp Val Val Ala His 145 150 155 160

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Page 62

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		aat Asn														146
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		cct Pro														242
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MBI-0021.txt

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Page 68

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